CAR lentiviral vector integration and T-cell outgrowth associated with formation of a novel CAR-MALT1 neomorphic allele

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CAR T therapy is an accepted alternative treatment to typical cancer treatments. During CAR T production, integration of vector DNA can disrupt genes, promote cell proliferation, and, in rare cases, potentially contribute to transformation. For HIV, insertional activation of genes such as STAT5A, MKL2, and BACH2 is associated with clonal expansion and persistence of the latent reservoir. Thus, studies of integration sites in patient samples, particularly from clonally expanded cells, may reveal genes or pathways influencing T cell persistence and function. For the case of CART therapy, such data can be valuable for optimizing CART therapy for HIV, cancer, and other disorders. Here we report unusual results from a patient who received a tandem product consisting of autologous CART19 and CART22 for B-cell leukemia and developed a severe immune response following treatment. Lentiviral integration site analysis of her T-cells revealed 74 distinct sense oriented integration sites in the first intron of MALT1, a paracaspase that plays a key role in T-cell receptor (TCR) mediated signaling. The transgene contained a cryptic splice donor, which we showed was spliced to form a coding region including both the CAR and MALT1 exon 2. Expression of the CAR-MALT fusion protein in cells was associated with constitutive MALT1 activation and cleavage of cellular MALT1 protein substrates, and this was associated with constitutive NF-kB pathway activation. Expression of the CAR-MALT1 fusion protein was lethal in primary cells and a Jurkat cell model, but lethality in the Jurkat cells was reversed by knocking out TRAF6, which mediates one branch of the MALT1 signaling pathway. Overexpression of MALT1 protein alone was not sufficient to activate MALT1 signaling, emphasizing the neomorphic nature of the CAR-MALT1 fusion protein. Smaller MALT1 integration site clusters were seen in two further CAR-T treated patients. We have so far not yet determined the mechanism of the differential responses among patients. We speculate that deleterious insertional mutagenesis events that would normally be negatively selected in vivo over time can in some situations be tolerated and thereby lead to unique
cell outgrowth and possible clinical effects.